

# Development and Evaluation of a Trapping System for *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in the United States

M. E. NEHME,<sup>1,2</sup> R. T. TROTTER,<sup>3</sup> M. A. KEENA,<sup>3,4</sup> C. MCFARLAND,<sup>5</sup> J. COOP,<sup>6</sup>  
H. M. HULL-SANDERS,<sup>7</sup> P. MENG,<sup>1</sup> C. M. DE MORAES,<sup>1,8</sup> M. C. MESCHER,<sup>1,8</sup> AND K. HOOVER<sup>1</sup>

Environ. Entomol. 43(4): 1034–1044 (2014); DOI: <http://dx.doi.org/10.1603/EN14049>

**ABSTRACT** *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), commonly known as the Asian longhorned beetle, is an invasive wood-boring pest that infests a number of hardwood species and causes considerable economic losses in North America, several countries in Europe, and in its native range in Asia. The success of eradication efforts may depend on early detection of introduced populations; however, detection has been limited to identification of tree damage (oviposition pits and exit holes), and the serendipitous collection of adults, often by members of the public. Here we describe the development, deployment, and evaluation of semiochemical-baited traps in the greater Worcester area in Massachusetts. Over 4 yr of trap evaluation (2009–2012), 1013 intercept panel traps were deployed, 876 of which were baited with three different families of lures. The families included lures exhibiting different rates of release of the male-produced *A. glabripennis* pheromone, lures with various combinations of plant volatiles, and lures with both the pheromone and plant volatiles combined. Overall, 45 individual beetles were captured in 40 different traps. Beetles were found only in traps with lures. In several cases, trap catches led to the more rapid discovery and management of previously unknown areas of infestation in the Worcester county regulated area. Analysis of the spatial distribution of traps and the known infested trees within the regulated area provides an estimate of the relationship between trap catch and beetle pressure exerted on the traps. Studies continue to optimize lure composition and trap placement.

**KEY WORDS** *Anoplophora glabripennis*, monitoring trap, trapping distance, male-produced pheromone, kairomone

Introduced and invasive species pose a serious threat to ecological and economic systems worldwide. In the United States alone, estimates of economic losses attributable to invasive species approach US\$120 billion per year (Pimentel et al. 2005). Forests in the north-eastern United States have particularly suffered from

exotic species invasions, some of which have severely altered ecosystem structure and function (Liebhold et al. 2013). Invasive species impacting this region include historically notable species and species complexes such as the pathogens responsible for Dutch elm disease and Chestnut blight, gypsy moth (*Lymantria dispar* (L.)), and winter moth (*Operophtera brumata* L.). More recently, the region has been impacted by the arrival and establishment of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), a cerambycid with a broad host range and the potential to dramatically alter the forested landscape of North America (Mack et al. 2000). This insect was recently listed among the 100 most threatening invasive species worldwide (Simberloff and Rejmanek 2011).

In its native range in China, Asian longhorned beetle primarily damages poplar tree species grown in timber plantations, posing a significant threat to 6.67 million hectares of poplar that account for ~20% of China's plantation-grown timber (Li et al. 2005) and causing economic losses estimated at US\$1.5 billion annually, which is nearly 12% of the total losses that are attributable to forest pests (Hu et al. 2009). In North America, Asian longhorned beetle was reported first in New York City in 1996, where it was likely

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service of any product or service to the exclusion of others that may be suitable.

<sup>1</sup> Department of Entomology and Center for Chemical Ecology, The Pennsylvania State University, 501 ASI Building, University Park, PA 16802.

<sup>2</sup> Lebanon Reforestation Initiative, 8th Floor Achrafieh Building 1020, Corniche Pierre Gemaue, Mathaf, Lebanon 00961 - 70 00 93 03.

<sup>3</sup> U.S. Forest Service Northern Research Station, 51 Mill Pond Road, Hamden, CT 06514.

<sup>4</sup> Corresponding author, e-mail: [mkeena@fs.fed.us](mailto:mkeena@fs.fed.us).

<sup>5</sup> USDA-APHIS, ALB Cooperative Eradication Program, 151 West Boylston Dr., Worcester, MA 01606.

<sup>6</sup> Massachusetts Department of Conservation and Recreation, ALB Cooperative Eradication Program, 151 West Boylston Dr., Worcester, MA 01606.

<sup>7</sup> USDA-APHIS-PPQ, Center for Plant Health Science and Technology, 1398 West Truck Road, Buzzards Bay, MA 02542-1329.

<sup>8</sup> Department of Environmental Systems Science, Swiss Federal Institute of Technology Zürich, LFO G 18, Schmelzbergstrasse 9, 8092 Zürich, Switzerland.

introduced in wood packaging material imported before 1990 (Bartell and Nair 2003, Government Accountability Office [GAO] 2006, Hu et al. 2009). Following its introduction and establishment, the beetle spread to an estimated 6,517 trees within a quarantined area of 36,777 hectares ( $\approx 142$  square miles) in the New York metropolitan area. Several additional breeding populations were subsequently identified in the United States, including one in Chicago, IL (1,771 trees in 9,583 quarantined hectares), two in New Jersey (729 trees in 6,475 quarantined hectares), and one in Bethel, OH (11,940 trees in 15,799 quarantined hectares). A new infestation (200 infested trees discovered so far) was also recently detected in Babylon Township, NY, and 7,252 additional hectares were added 16 April 2014 to the New York quarantine area (U.S. Department of Agriculture–Animal Plant Health Inspection Service [USDA–APHIS] 2013a, 2014a). However, by far the largest known North American population of Asian longhorned beetle was discovered in Worcester, MA, in 2008 (USDA–APHIS 2014b).

Establishment of Asian longhorned beetle populations in the greater Worcester area presents serious challenges to eradication programs. The discovery of Asian longhorned beetle in Worcester, MA, in 2008 led to the establishment of a 73 square mile (18,910 ha) regulated area in early 2009 (Baca et al. 2009) and since then the continued discovery of new infestations has expanded this regulated area (Markham and Reardon 2013). Beyond the sheer size of the infestation ( $>23,700$  infested trees in 28,490 quarantined hectares as of May 2014), the beetle has established in an area that transitions directly from urban to forested landscapes and contains a large proportion of susceptible host trees: red maple is the second most abundant (based on volume) tree species in Massachusetts, whereas sugar maple ranks fifth and birch ranks eighth (Butler et al. 2012). This landscape structure and host availability highlights the need for effective management of beetle populations in this region.

In an effort to eradicate this pest, local, state, and federal agencies have surveyed  $>3.9$  million trees in the Massachusetts townships of Worcester, Boylston, West Boylston, Shrewsbury, Holden, and Auburn. As of May 2014, 23,586 infested trees had been detected and removed, and nearly 200,000 trees have been treated with the systemic insecticide imidacloprid (USDA–APHIS 2014b). Surveys for infested trees and signs of beetle presence have been conducted primarily by tree climbers and ground surveyors using binoculars, methods that are both costly and time consuming. In addition, ground surveys are estimated to be only 30% effective at spotting infested trees when only oviposition sites are present, while even tree climbers are only  $\approx 60$ –75% effective (USDA–APHIS, 2013b). Consequently, the development of more efficient and cost-effective methods for the detection of infestations and for monitoring and verification of eradication programs is a top priority. To this end, the potential use of semiochemicals as trapping lures for Asian longhorned beetle has been investigated for

several years (Zhang et al. 2002; Hu et al. 2009; Nehme et al. 2009, 2010).

The identification of potentially attractive semiochemicals should be informed by an understanding of the ecology and natural history of the beetle. Asian longhorned beetle has been described as a somewhat sedentary species, which often reinfests the same host tree until it is exhausted as a resource (Williams et al. 2004). Mate finding and copulation appear to involve a complex series of behaviors, including responses to chemical and visual cues. Males produce a volatile pheromone (Zhang et al. 2002) that, when perceived in combination with certain plant-derived volatile compounds, attracts primarily virgin females (Nehme et al. 2010). Once males and females are in proximity, mate finding appears to include additional visual and chemical cues, including a female-produced sex trail pheromone (laid down by the female as she walks across the host) that is attractive only to males (Hoover et al. 2014) and a female-produced contact pheromone that stimulates males to initiate mating (Zhang et al. 2003).

In earlier field studies conducted in China, Nehme et al. (2010) reported that a mixture of the plant volatiles linalool, linalool oxide, *cis*-3-hexen-1-ol, *trans*-pinocarveol, and *trans*-caryophyllene, presented in combination with the male pheromone—a 1:1 mixture of 4-(*n*-heptyloxy)butan-1-ol and 4-(*n*-heptyloxy)butanal—significantly increased trap catches of females, of which 85% were found to be virgins. The same study evaluated several different trap designs and found Intercept panel traps to be the most practical and effective (Nehme et al. 2010).

Building on this work, a series of seasonal trapping studies were initiated from 2009 to 2012 to evaluate and improve lure efficacy in the invasive Asian longhorned beetle population in Massachusetts. Traps were deployed throughout the Worcester area with the primary goal to determine whether semiochemical-based traps can successfully capture beetles in both an urban and forested landscape in the eastern United States. At the same time various lures were evaluated and the potential distance in one season that a beetle will travel from an infested tree to a trap was estimated under field conditions.

## Materials and Methods

The trapping area is under both federal and state quarantine, and the mission of the USDA–APHIS program is eradication. This meant that the number of trapped beetles would be expected to decrease, as the eradication program removed beetle-infested trees over time.

**Trap Design and Deployment.** Intercept panel traps (forestry panel traps; Alpha Scents, Syracuse, NY) with collecting cups attached by zip ties were used from 2009 to 2011. In 2012, damaged traps were replaced with woodborer panel traps (ChemTica Internacional S.A., Heredia, Costa Rica), and all traps were retrofitted with locking twist off collection vessels (ChemTica Internacional S.A. modification). In 2009,

traps were coated with Rain-X (ITW Global Brands, Houston, TX) to increase the slipperiness of the trap surface. In 2010, a mixture of Fluon (Northern Products Inc., Dudley, MA) and 5% India ink (Graham et al. 2010), to retain the black color of the trap, was used instead of Rain-X. In 2011–2012, the traps were coated with a diluted Fluon solution (10% vol:vol), and the India ink was omitted because the diluted solution did not alter trap color. Traps were dismantled and cleaned each year and recoated with that year's antitraction coating. The switch to Fluon was based on the findings of Graham et al. (2010), who reported that this treatment increased trap catches of cerambycids by >14% compared with traps coated with Rain-X (Graham et al. 2010).

In 2009, tree climbers installed each trap by selecting a limb near the base of the tree canopy and installing a rope pulley system, attached to the tree with rings and hooks. The free end of the rope was attached to a hook set into the tree trunk  $\approx 2$  m above ground. This approach was intended to minimize public access to the traps, while allowing project personnel, using a ladder, to lower the traps for checking. In subsequent years, a simplified pulley system, consisting of paracord hung over a tree limb, was used to suspend traps out of the reach of the public, reducing the time required to deploy and service traps. The free end of the paracord was secured to the trunk with a UV-stable zip tie. Traps were suspended from limbs  $\approx 5$  m above the ground that appeared sturdy and either angled upwards or possessed side branches to prevent the paracord from slipping off. Deployed traps did not directly contact foliage or other limbs. Where possible, susceptible open-growing or forest-edge trees were selected for trapping so that beetles could easily fly into the traps and because the beetle is known to inhabit riparian edges in forests in its native habitat (Williams et al. 2004). Nonhost tree species near *Acer* spp. were sometimes used when no other option was available in a given location. Trap height, tree species, diameter at breast height (DBH) and latitude/longitude coordinates were recorded for each tree. To kill and preserve trapped insects, the trap collection vessels contained  $\approx 3$  cm of a 5% propylene glycol solution (antifreeze, purchased locally) in 2009–2010, and a saturated salt solution in 2011–2012. Traps were checked every other week, and the solution in the vessels was replaced. All invertebrates caught in the traps were collected, placed in plastic bags, and were frozen until assessed. Damaged specimens were counted, identified where possible and then discarded. Specimens in good shape were counted, separated by order and stored in alcohol or pinned for further analyses (not presented here). Voucher specimens of beetles from Worcester, MA (not the ones trapped because they were dissected), have been deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT. Appropriate permissions were obtained to place traps on private, city, and state lands.

**Trap Distribution.** A spatial overview of the distribution of traps in the greater Worcester area, along

with the locations of documented infested trees (through December 2012), is provided in Fig. 1 and Supp Fig. 1 [online only]. In 2009, 82 traps were deployed across the regulated area during 16 June–20 and removed during 15 November–20. In 2010, 40 traps were deployed during 1 June–2, at nine locations, with three to five traps at each location, and removed on 6 October. In 2011, 500 traps were deployed during 4 July–17 in ten 5-km transects, each with 50 traps spaced 100 m apart. These transects were placed in areas where infested trees had previously been detected and removed as well as in unsurveyed areas. All traps were removed during the last week of September. In 2012, 391 traps were deployed in lines between 18 June and 3 July, and placed 100 m apart in areas near recent infested tree finds, areas between the previous year's transects, and areas not yet surveyed. Traps were removed during 16 September–19.

**Lures.** The lures used for these studies included various mixtures of the plant volatiles linalool, linalool oxide, *cis*-3-hexen-1-ol, *trans*-pinocarveol,  $\delta$ -3-carene, and *trans*-caryophyllene presented alone or in combination with the Asian longhorned beetle male-produced pheromone, which is a 1:1 mixture of 4-(*n*-heptyloxy)butan-1-ol and 4-(*n*-heptyloxy)butanal (Zhang et al. 2002). Beginning in 2012, *trans*-pinocarveol was dropped as a plant volatile component based on results of the 2009 and 2010 trapping and laboratory studies conducted in 2011 (M.E.N., unpublished data). Table 1 presents the number of traps used by lure family, lure type, and year. Resources and sample sizes did not allow for the analysis of all possible lure combinations. The combinations that were selected were based on previously published data, and concurrent research going on in China in a separate study designed to evaluate some of these combinations. The lure formulations and emitters were prepared by either ChemTica (ChemTica Internacional S.A., Heredia, Costa Rica) or Synergy (Synergy Semiochemicals Corp., Burnaby, British Columbia, Canada). The lure suppliers were based on the ability of the company to produce lures to given specifications in the required time frames, and potential differences were not evaluated because the study lacks adequate sample sizes. ChemTica emitters consisted of plastic pouches with color-coding for each chemical, and Synergy emitters consisted of clear plastic bubbles with colored liquid to distinguish the chemicals. Lures were initially assigned randomly in 2009, changed monthly, and rotated sequentially between the traps within each site every month. In 2010, the lures were assigned randomly and changed monthly, but not rotated. In 2011 and 2012, the lure treatments were deployed in a repeating pattern to allow evaluation of potential between-lure effects in each transect or line. In 2011, every 10th trap served as an unbaited control and the control was part of the repeating pattern in 2012.

To monitor release rates of pheromone and kairomone components, beginning in 2011 one lure of each component was hung outdoors at the USDA Forest Service Northern Research Station in Hamden, CT (a

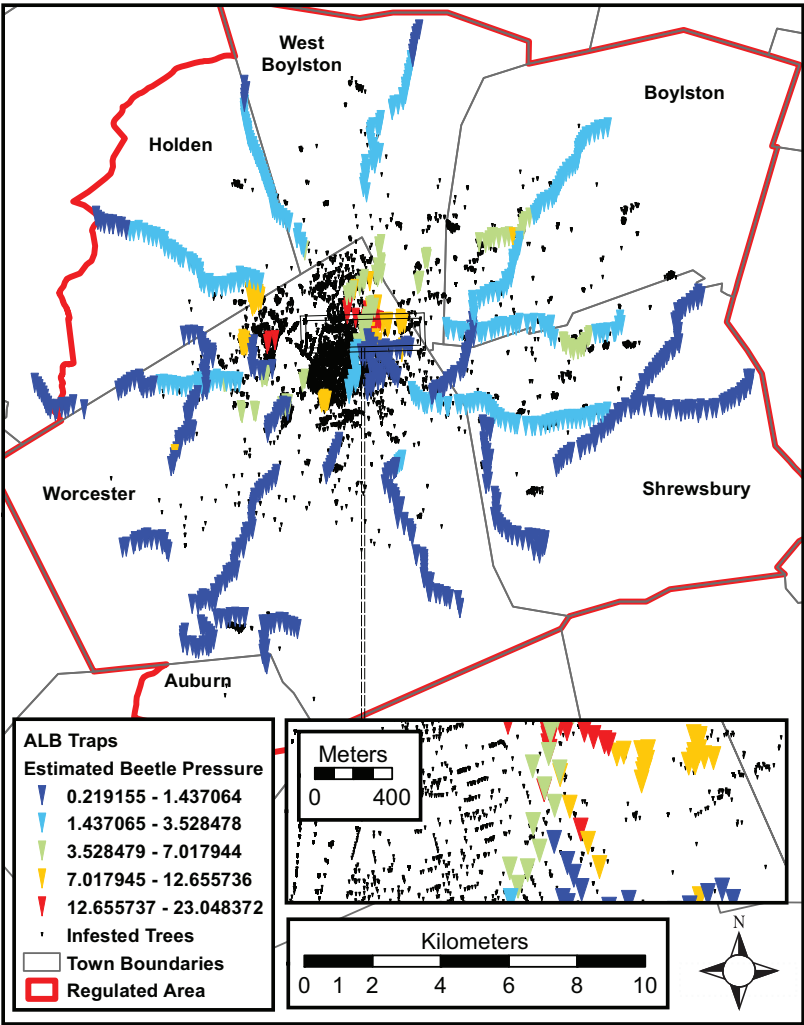


Fig. 1. Filled colored triangles (many overlapping each other) indicate the locations of individual traps, and the estimated beetle pressure on that trap at the time of its deployment. Small black triangles (also many overlapping) indicate the locations of know infested trees that were removed soon after they were found. The perimeter of the regulated area is shown as a red line. Inset shows detail of area where many infested trees were present and beetle pressure on placed traps was high.

location exhibiting similar climatic conditions as Worcester), and lures were weighed weekly. In 2011, two components, 4-(*n*-heptyloxy)butanal and *trans*-caryophyllene, were found to be releasing at undesirably rapid rates—leading to deviation from the targeted blend ratios—and were subsequently placed in air-tight polyethylene pill pouches (Walgreens Co., Deerfield, IL) to slow their release (values given in Table 1 are the reduced rates following the addition of the polyethylene pouches). New lures were added to traps when components neared depletion to maintain adequate release rates throughout the season. In 2012, based on the Hamden monitoring, a second emitter each of 4-(*n*-heptyloxy)butanal and *trans*-caryophyllene was added to traps with lures containing these components after 4 wk and all lures were replaced at 8 wk.

The release rates of three plant volatiles, *cis*-3-hexen-1-ol, *trans*-caryophyllene, and linalool, were adjusted in 2012 to approximate the ratios detected in volatile extractions of 3-yr-old, potted *Acer pictum* var. *mono* (Maximovich), known to be highly attractive to Asian longhorned beetle adults (Zhang et al. 2007). Volatile collections from these greenhouse-grown saplings were conducted in the summer of 2011 to determine the identity and calculate the relative ratios of the compounds. The top five leaves (amount of tissue standardized but not quantified) still attached to the trees were placed in closed 4-liter glass-dome volatile collection chambers with Teflon Bases that closed around the plant stem. Charcoal-filtered air was pushed into the chamber and air was pulled out through a Super-Q filter (4-mm-diameter glass tube containing 45 mg Super-Q [Alltech, Deerfield, IL]) at

Table 1. Overview of the number of traps used by lure family, year, and lure type

Lure family code	Year	Lure	Male pheromone (mg/d) <sup>a</sup>			Plant volatiles (mg/d) <sup>a</sup>					Beetles caught	Traps with beetles	Total traps
			Combined	Alcohol	Aldehyde	Linalool	<i>cis</i> -3-Hexen-1-ol	Linalool oxide	Caryophyllene	<i>trans</i> -Phocarbeol	3-Carene		
1	2009	A	0.01								0	0	8
1	2009	B	0.1								0	0	8
1	2009	C	1.0								1	1	8
1	2012	M		1.0 (0.7)	1.0 (1.0)						2	2	66
1	2012	N		5.0 (3.4)	5.0 (5.0)						0	0	65
2	2009	D	0.01			1.0	1.0	1.0	1.0	1.0	6	4	14
2	2009	E	0.01			1.0	1.0	1.0	1.0		1	1	16
2	2010	G	0.1			3.0	1.0	1.0	4.0	1.0	3	2	20
2	2010	H	0.1			3.0	1.0	1.0	4.0		1	1	10
2	2011	I		1.0 (0.5)	1.0 (2.5)	4.0 (3.5)	1.0 (1.0)	1.0 (1.0)	5.0 (16)		6	5	112
2	2011	J		1.0 (0.5)	1.0 (2.5)	4.0 (3.5)	1.0 (1.0)		5.0 (16)		8	7	113
2	2011	K		5.0 (2.0)	5.0 (12)	4.0 (3.5)	1.0 (1.0)	1.0 (1.0)	5.0 (16)		4	4	112
2	2011	L	0.1			3.0	1.0	1.0	4.0		5	5	113
2	2012	O		1.0 (0.7)	1.0 (1.0)	8.0 (6.3)	1.0 (1.2)		9.0 (8.3)		2	2	65
2	2012	P		5.0 (3.4)	5.0 (5.0)	8.0 (6.3)	1.0 (1.2)		9.0 (8.3)		4	4	65
3	2009	F				1.0	1.0	1.0	1.0		2	2	16
3	2012	Q				8.0 (6.3)	1.0 (1.2)		9.0 (8.3)		0	0	65
4	2009	Cntl									0	0	12
4	2010	Cntl									0	0	10
4	2011	Cntl									0	0	50
4	2012	Cntl									0	0	65

<sup>a</sup> Nominal release rates (mg/d) are given with estimated average release rates (mg/d) at 25°C in parentheses below, if known.

Lure family codes: 1, male pheromone alone; 2, male pheromone in combination with plant volatiles; 3, plant volatiles alone; 4, no lure (control).

All lures used in 2009, 2010, and 2012 were produced by ChemTica Internacional S.A. All the emitters for 2011 were produced by Synergy Semiochemicals Corp. (Burnaby, BC, Canada) except for the kairomone emitters for lure "L," which were produced by ChemTica Internacional S.A. in 2010.



a push-pull flow rate = 3.0–1.5 liters per min (De Moraes and Mescher 2004). The high air flow into the domes and use of only one collection filter at a time was done to avoid condensation and water accumulation in the domes. Volatiles were collected over three 5-h intervals during a 24-h period (0800–1300, 1320–1820, and 2200–0300). The Super-Q filters were changed after each 5-h collection. This process was replicated with six healthy *Ac. mono* saplings of the same age. Simultaneous collections from empty chambers provided a control for atmospheric contaminants.

Samples were eluted from the Super-Q traps using 150  $\mu\text{l}$  of dichloromethane ( $\text{CH}_2\text{Cl}_2$ ), and 10  $\mu\text{l}$  of an internal standard (20 ng/ $\mu\text{l}$  nonyl acetate and 10 ng/ $\mu\text{l}$  octane; Sigma-Aldrich, St. Louis, MO) was added. Aliquots (2  $\mu\text{l}$ ) of samples collected as described above were injected into an Agilent 6890 gas chromatograph (GC; Agilent Technologies, Palo Alto, CA) fitted with a flame ionization detector (FID) or an Agilent 5973 mass spectrometer (MS) in the splitless mode for 0.3 min. A Vocol capillary column of intermediate polarity (30 m  $\times$  0.25 mm i.d., 1.50  $\mu\text{m}$  film thickness; Supelco, Bellefonte, USA) was used for GC-FID and GC-MS analyses. Compounds were identified by matching against NIST 2005 library standards. Quantification reports were thus generated for each sample, and production times were compared. Because saplings produced more volatiles during the first collection period (0800–1300), the six replicates from that period were used to determine the ratios to be used in the trapping. The volatiles collected were corrected against the blank control and averaged across the trees for quantities of each volatile produced during this collection period to obtain the final ratios. The three compounds were produced by *A. pictum* var. *mono* at an average of 83.72, 97.71, and 11.42 ng in a 5-hr time period, respectively. Based on this finding, a simplified ratio of *cis*-3-hexen-1-ol:linalool:*trans*-caryophyllene of 1:8:9 was used in the lures during our 2012 trapping season.

**Statistical Analyses.** To determine whether baited traps were effective in attracting Asian longhorned beetle, the numbers of control and baited traps that caught any beetles were compared using a  $\chi^2$  test (CHISQ.TEST in Excel V14.0.6129.5000). Analyses were conducted from a trap-centric perspective (did a trap catch beetles), rather than a beetle-centric perspective (how many beetles in a trap) for two reasons. First, this approach does not require the assumption of independence among individual beetles, and avoids bias that could be introduced by intertrap variation in beetle pressure. Second, only five of the 40 traps collected multiple beetles, and each of these traps collected two beetles (45 beetles in 40 traps), and so the loss of analytical sensitivity is limited. A post hoc comparison of relative beetle pressure on both control and baited traps was run to determine the potential for bias in trap success due to variation in the abundance and distribution of beetles based on the current (as of 27 June 2013) ALB Eradication Program database, which included full records for  $\approx 14,500$  individual trees. Beetle pressure on each trap was cal-

culated using three parameters: 1) the distances between each trap and each infested tree; 2) the timing of trap placement and tree removal (i.e., trees were filtered such that trees removed before the date of trap placement were not included); and 3) the level of infestation in each tree. Trap tree distances were calculated using Point Distance in ArcMap 10.0 (Service Pack 4, Environmental Systems Research Institute [ESRI] 2010). The inverse of each distance ( $D$ , to adjust for diffusion) was then multiplied by the infestation weight of the tree ( $W$ ). Infestation weights were determined by the cooperative eradication program, with each tree being assigned to one of four infestation levels (A, B, C, D); A-trees had oviposition pits, but no exit holes, B-trees had 1–10 exit holes, C-trees had 11–100 exit holes, and D-trees had >100 exit holes. Numeric weights of 1, 10, 100, and 1,000 were applied to tree levels A, B, C, and D, respectively, to reflect the log-scale density of beetles implicit in the infestation levels. Tree records were filtered to remove pressure imposed by trees that were removed before a trap was placed, and the total beetle pressure ( $P$ ) exerted by all included trees ( $t$ ) was summed for each trap ( $n$ ) such that

$$P_n = \sum_t W_t (1/D). \quad [1]$$

Beetle pressures were calculated using a custom-built routine (WeightCalculations.m) in MatLab (R2012b, The Mathworks Inc. 2012). Cumulative distributions of beetle pressure among trap groups were compared using a Kolmogorov–Smirnov test (ks.test) in R (V2.15.3, R Core Team 2012).

To determine trap efficacy, the probability of a trap collecting a beetle at a given beetle pressure was estimated by fitting a logistic regression using the GLM function in R (V2.15.3, R Core Team 2012).

## Results

From 2009 through 2012, 45 *A. glabripennis* beetles were captured in 40 traps baited with the male beetle pheromone, plant volatile mixtures, or a combination of the two; no control trap collected a beetle (Table 2). Analyses of these data via  $\chi^2$  confirmed a statistically significant effect of the lures (taken as a whole) on beetle attraction ( $\chi^2 = 6.51$ ; df = 1;  $P = 0.01071$ ). As might be expected, beetle pressure was a significant predictor of the likelihood of an individual trap collecting a beetle, (Fig. 2; z value [intercept] =  $-16.74$ ,  $P < 0.0001$ ; z value [beetle pressure] =  $3.97$ ,  $P < 0.0001$ ). A post hoc comparison of the beetle pressure exerted on baited and unbaited traps shows that the pressure exerted on the two groups did not differ (Kolmogorov–Smirnov  $D = 0.1071$ ,  $P = 0.1317$ ), eliminating the alternative hypothesis that trap catch differences between the two groups resulted from differences in ambient beetle pressure on the traps.

The reduction in the number of beetles collected by traps through time is consistent with the reduction in the overall number of beetles found in the regulated area over the course of the study (Fig. 3). Figure 3

Table 2. Number of beetles caught by trap type

Treatment	Total number of traps	Observed		Expected	
		No. of traps with beetles	No. of traps without beetles	No. of traps with beetles	No. of traps without beetles
Baited	876	40	836	34.6	841.4
Control	137	0	137	5.4	131.6
Total	1,013	40	973	40	973

$\chi^2 P < 0.01071$ .

shows the number of infested trees detected and removed by year (based on those trees for which full records are available), and compares it with the percentage of traps with lures that caught beetles. Because the eradication program has aggressively surveyed for and removed infested trees, the number of beetles found in Worcester County has decreased annually. It is interesting to note, however, that while the total number of beetles found each year has decreased, the proportion of those beetles found using traps has increased.

Over the 4 yr of the study, 17 different lure compositions were tested (in addition to a blank control lure). Ongoing work on these combinations, including via field studies in China where higher beetle densities permit more rigorous investigation of questions about optimal lure design, suggests that some are more effective than others in attracting beetles (Nehme et al. 2010; Meng 2014). The relatively small number of beetles captured in the current study does not allow comparison of the relative efficacy of individual lures; however, the lures can be broadly grouped into three families: 1) those with male beetle pheromone only, 2) those with plant volatiles only, and 3) those with both the male pheromone and plant volatiles. The distribution of beetles captured by these lure families is shown in Table 1. From these groupings, the trapping data suggest a trend toward greatest attraction to lures combining the beetle pheromone and plant volatiles; however, this trend was not statistically significant

( $\chi^2 = 4.48$ ;  $df = 2$ ;  $P = 0.1067$ ), and further work, perhaps in areas with greater beetle density, will be necessary to identify potential differences in lure efficacy.

The average height at which traps were placed was  $5.5 \pm 0.1$  m (range, 1.6–16.2 m); traps that caught beetles were hung at  $5.2 \pm 0.2$  m (range, 2.5–8.2 m) off the ground. The average DBH of the trees the traps were hung in was  $50.4 \pm 0.8$  cm (range, 3.0–200 cm) and trees with traps that caught beetles were  $49.6 \pm 3.4$  cm DBH. Seventy-four percent of the traps were hung on *A. glabripennis* host trees (70% on *Acer* species), and these traps accounted for 90% of the beetles collected. Four of the beetles trapped in 2011 were caught in traps hung on nonhost trees, including choke cherry (*Prunus virginiana* L.), shagbark hickory (*Carya ovata* (Miller) K. Koch), and white oak (*Quercus alba* L.; two beetles). The two males that were trapped in 2011 were caught on *Quercus* spp. and *Acer* spp.

Figure 4 provides a summary of the distributions of distances between traps that collected beetles, and the nearest known infested tree present at the time the trap was deployed, which may be useful for determining the best spacing of traps. Although the minimum distances shown (some <20 m) suggest the beetles may have come from nearby (detected) infestations, many of the distances exceeded 1 km, suggesting the likelihood of nearer, as-yet undetected, sites of infestation. The effects of wind speed, direction, and topography are also likely to interact, modifying the distances at which beetles may detect lures in a field setting.

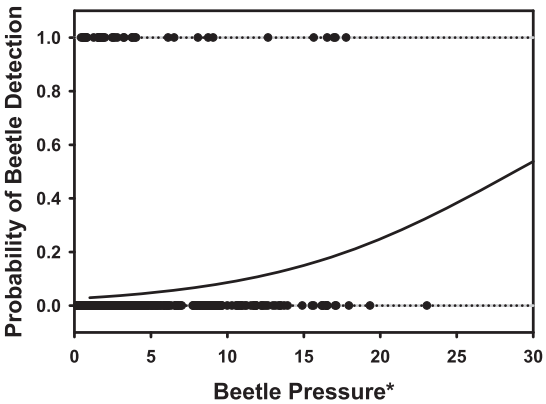


Fig. 2. Points represent traps with and without beetles along the beetle pressure gradient. The line shows the relationship between the probability of a trap collecting a beetle and the beetle pressure acting on the trap. \*Based on equation 1.

Discussion

The current findings show that traps baited with male beetle pheromones, presented alone or in combination with plant-derived volatile compounds, can be effectively deployed in invaded landscapes to detect beetles. Indeed, the trapping efforts described here led in some cases to the detection of previously undiscovered beetle infestations in areas that had not yet been surveyed (for example, in Shrewsbury, MA, east of the core of the infestation in Worcester in 2011). Increasing the speed of detection, thus reducing the time available for beetle population growth before the initiation of control efforts, has major implications for management. And the use of trapping data to prioritize areas for tree climbing surveys and

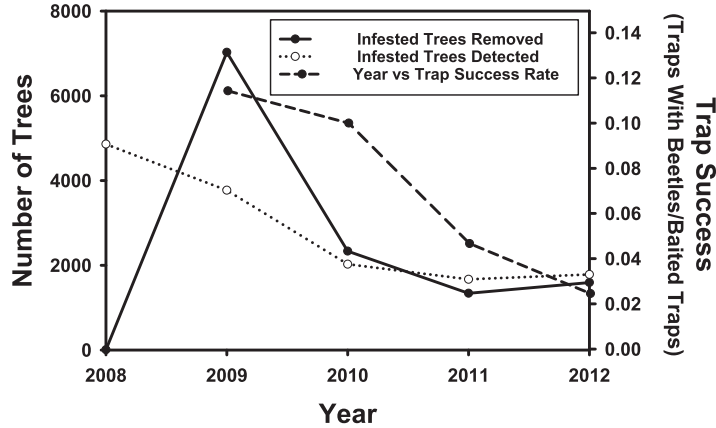


Fig. 3. The number of trees detected and removed has declined since the first documentation of the infestation in 2008, suggesting the removal of infested trees from the landscape is associated with a decrease in trapping success (defined as the number of traps with beetles/number of baited traps).

other interventions appears to have significant potential in this regard.

Traps may also prove useful as a tool for detecting the presence of infested trees in previously surveyed areas. In several cases over the course of the 4-yr study, trap captures guided the eradication team to trees with cryptic infestations that had not been detected in earlier surveys. For example, in 2009, two beetles were trapped in Dodge Park, an area where Asian longhorned beetle initial surveys had been completed, and on Doyle Street where host trees are scarce and scattered. Guided by these trap catches, two new infested trees were found in Dodge Park. Similarly, in 2010 and 2011, four females were trapped in baited traps hung on the same tree on Lansing Avenue near Indian Lake and finally after surveying the area both years after the beetles were trapped, a small boxelder across the street was discovered that is thought to be the source of the beetles. Furthermore, the identification of some of these infested trees triggered expansion of the regulated boundary to the west. (It is worth noting, how-

ever, that in some cases, the source of beetles found in our traps has not been determined despite targeted surveys in the surrounding areas).

This current study complements and provides a check on the applicability of the findings of both previous and current work in China where higher beetle densities facilitate investigation of lure composition. Earlier studies conducted in higher beetle density areas in China indicated a combination of male-produced pheromone and plant volatiles were the most effective lures for Asian longhorned beetle (Nehme et al. 2010). These findings are consistent with the overall pattern observed in the current study, though the relatively small number of beetles caught prevents the statistical support of this pattern. There is also evidence from other systems that female cerambycids use male-produced pheromones and plant volatiles for mate location (Ginzel and Hanks 2005, Reddy et al. 2005). Although the current findings show that baited traps can be effective in trapping beetles in an invaded landscape, more work remains to be done to optimize the lures used.

The effective use of traps in a management context can be advanced by quantifying the relationship between beetle population density, and the probability of beetle detection by trapping. This relationship could in turn be used to generate confidence intervals for the probability of confirming the absence of beetles (or populations below some threshold) in a given population by deploying a set number and density of traps. However, this link will depend on the development of more precise estimates of beetle population densities in infested areas than what is currently available in the United States, and may require the evaluation of traps in regions with native infestations of the beetle such as China (Meng 2014). The current data suggest beetles were collected when traps were within 3–366 m of an infested tree (Table 3). It should be noted that these distance estimates can only provide a distance to the nearest possible tree the beetle may have last visited to chew an oviposition pit or an exit

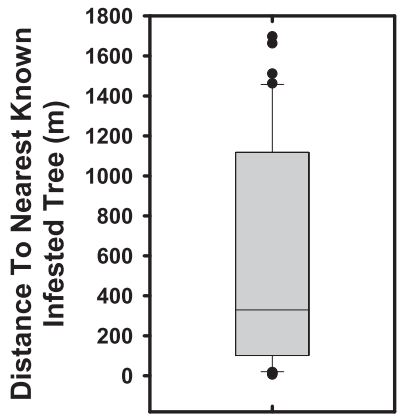


Fig. 4. The distribution of distances between traps that collected beetles and trees known to be infested and present at the time of the deployment of the trap.



Table 3. Information on each trap that caught beetles sorted by tree species the trap was hung on within each lure

Trap ID	No. of Asian longhorned beetle caught	Year	Lure	Lure family	Tree species	Trap height (m)	Distance to nearest infested tree (m) and infestation code	Beetle pressure
10	1F	2009	C	1	<i>Acer platanoides</i> L.	N/A	6 A	16.54
2	2F	2009	D	2	<i>Acer platanoides</i>	N/A	7 A	17.77
3	1F	2009	D	2	<i>Acer platanoides</i>	N/A	3 B	15.65
11	1F	2009	D	2	<i>Acer platanoides</i>	N/A	17 A	16.97
6	2F	2009	D	2	<i>Acer rubrum</i> L.	N/A	29 A	8.08
14	1F	2009	E	2	<i>Acer rubrum</i>	N/A	13 A	17.08
20	1F	2009	F	3	<i>Acer saccharum</i> Marshall	N/A	48 A	12.66
7	1F	2009	F	3	<i>Acer saccharum</i>	N/A	65 U	9.08
16	1F	2010	G	2	<i>Acer platanoides</i>	5.56	63 U	6.52
42	1F	2010	G	2	<i>Acer rubrum</i>	2.53	216 A	6.12
29	1F	2010	H	2	<i>Acer saccharinum</i> L.	5.14	87 B	8.74
293	1F	2011	I	2	<i>Acer platanoides</i>	7.24	723 A	0.81
484	1F	2011	I	2	<i>Acer platanoides</i>	4.48	6 A	3.78
493	2F	2011	I	2	<i>Acer platanoides</i>	5.43	8 A	2.80
497	1F	2011	I	2	<i>Acer platanoides</i>	3.33	31 A	2.46
168	1M	2011	I	2	<i>Quercus alba</i>	3.94	228 B	1.88
451	1M	2011	J	2	<i>Acer platanoides</i>	8.11	100 U	4.02
144	1F	2011	J	2	<i>Acer platanoides</i>	6.89	74 A	3.21
194	1F	2011	J	2	<i>Acer platanoides</i>	3.23	18 A	1.56
301	2F	2011	J	2	<i>Acer platanoides</i>	5.74	4 A	1.99
64	1F	2011	J	2	<i>Acer saccharum</i>	4.78	130 B	3.76
197	1F	2011	J	2	<i>Acer rubrum</i>	7.16	24 A	1.56
411	1F	2011	J	2	<i>Prunus virginiana</i>	2.57	366 A	2.64
165	1F	2011	K	2	<i>Acer platanoides</i>	4.05	14 B	1.86
308	1F	2011	K	2	<i>Acer saccharum</i>	8.23	6 A	1.79
178	1F	2011	K	2	<i>Carya ovata</i>	4.94	250 U	1.62
65	1F	2011	K	2	<i>Quercus alba</i>	7.32	99 A	3.93
179	1F	2011	L	2	<i>Acer platanoides</i>	4.57	266 A	1.64
193	1F	2011	L	2	<i>Acer platanoides</i>	6.07	68 A	1.55
489	1F	2011	L	2	<i>Acer platanoides</i>	4.57	6 B	3.25
496	1F	2011	L	2	<i>Acer platanoides</i>	5.40	3 A	2.54
176	1F	2011	L	2	<i>Acer saccharinum</i>	4.37	41 B	1.69
353	1F	2012	M	2	<i>Acer platanoides</i>	6.07	76 U	0.43
105	1F	2012	M	2	<i>Acer rubrum</i>	4.61	17 A	0.81
374	1F	2012	O	2	<i>Acer rubrum</i>	3.85	5 A	0.59
88	1F	2012	O	2	<i>Acer saccharum</i>	5.14	24 A	0.66
86	1F	2012	P	2	<i>Acer platanoides</i>	4.65	45 A	0.64
365	1F	2012	P	2	<i>Acer platanoides</i>	3.24	37 A	0.56
376	1F	2012	P	2	<i>Acer rubrum</i>	5.44	34 U	0.61
125	1F	2012	P	2	<i>Acer saccharum</i>	6.46	181 B	1.26

Infestation codes: U, unknown; A, oviposition pits only; B, 1–10 exit holes.

hole, but does not define the distance at which a beetle will detect and orient toward the lure or how far it will travel in a season. It should also be noted that estimates of effective distances are limited by the relatively low number of beetle captures, the potential effects of wind direction and velocity, the potential for human-aided movement (e.g., moving infested wood or beetles riding on vehicles), and because not all infested trees on the landscape are known.

Beetle abundance within the regulated area appears to have declined over the course of the study. A comparison of the beetle pressure exerted on traps by year (Table 3) suggests beetle pressure on these traps decreased each year, concomitantly with reductions in overall beetle trap-catch through time. Detections of beetles by other means agree with this trend. The eradication program keeps records on total beetles collected (by residents, ground surveyors and climbers, and trapping) in the regulated area. Of 29 adult beetles found in Worcester in 2009, 10 were caught in traps. Of 176 beetles collected in 2010, four were caught in the traps despite few traps being deployed. Of the 35 beetles collected in 2011, 23 were caught in

the traps. Of 13 beetles collected in 2012, eight were caught in the study traps, and an additional beetle was caught in a MA Department of Conservation and Recreation trap (baited using one of the experimental lures evaluated in this study).

The protection of forests in North America depends on the prevention of exotic species introductions, the rapid detection of infestations of invasive species that do become established, and the effective eradication of established populations. Our findings demonstrate the potential value of fly-in panel traps baited with appropriate olfactory cues as a tool for identifying new or recurrent infestations of Asian longhorned beetle and as a means of prioritizing areas for the deployment of scarce management resources. Continued refinement of trapping methods, including through efforts to optimize the composition and release rates of blend components, should further enhance the value of these methods for management efforts aimed at Asian longhorned beetle, and also provide a framework for future efforts to manage other invasive insect pests.

## Acknowledgments

We thank B. Aflague, K. Benkoski, G. Bradford, J. Birosack, P. Elder, C. Fagan, J. Franklin, A. Hoover, D. Long, J. McNeil, D. Mikus, W. Panagakos, V. Pavao, C. Perry, D. Roberts, A. Roth, V. Sánchez, E. Schwartzberg, B. Secskas, and M. Shaiyen for technical support in setting up and servicing the traps. We thank K. Freeman, R. Mack, A. Sawyer, C. Markham, D. Lance, V. Mastro, and the survey crew at the ALB Eradication Program Office in Worcester, MA, for assistance with obtaining information on infested trees or help in surveying around positive traps. This work was supported by grants to K.H. from the Alphawood Foundation, the Horticultural Research Institute, and cooperative agreements with the USDA, APHIS, PPQ (Plant Protection and Quarantine), and USDA Forest Service, State and Private Forestry Northeastern Area, Technology & Methods Development 10-CA-11420004-316.

## References Cited

- Baca, R. M., J. Twardowski, and C. Markham. 2009. Asian longhorned beetle successes and challenges in 2008, pp. 5–6. In K. A. McManus and K. W. Gottschalk (eds.), Proceedings of the 20th U.S. Department of Agriculture interagency research forum on invasive species 2009, January 13–16, Annapolis, MD. Gen. Tech. Rep. NRS-P-51. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.
- Bartell, S. M., and S. K. Nair. 2003. Establishment risks for invasive species. *Risk Anal.* 24: 833–845.
- Butler, B. J., R. S. Morin, M. D. Nelson. 2012. Massachusetts' forest resources, 2011. Res. Note NRS-150. U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA: p. 4.
- De Moraes, C. M., and M. C. Mescher. 2004. Biochemical crypsis in the avoidance of natural enemies by an insect herbivore. *Proc. Natl. Acad. Sci. U.S.A.* 101: 8993–8997.
- (ESRI) Environmental Systems Research Institute. 2010. ArcMap ArcInfo version 10.0. Environmental Systems Research Institute, Inc., Redlands, CA.
- (GAO) Government Accountability Office. 2006. Invasive forest pests: lesson learned from three recent infestations may aid in managing future efforts. Report from the United States Government Accountability Office to the Chairman, Committee on Resources, House of Representatives. GAO-06–353.
- Ginzel, M. D., and L. M. Hanks. 2005. Role of host plant volatiles in mate location for three species of longhorned beetles. *J. Chem. Ecol.* 31: 213–217.
- Graham, E. E., R. F. Mitchell, P. F. Reagel, J. D. Barbour, J. G. Millar, and L. M. Hanks. 2010. Treating panel traps with a fluoropolymer enhances their efficiency in capturing cerambycid beetles. *J. Econ. Entomol.* 103: 641–647.
- Hoover, K., M. A. Keena, M. E. Nehme, S. Wang, P. Meng, and A. Zhang. 2014. Sex-specific trail pheromone mediates complex mate finding behavior in *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *J. Chem. Ecol.* 40: 169–180.
- Hu, J., A. Sergio, S. Schuetz, Y. Luo, and A. E. Hajek. 2009. Ecology and management of exotic and endemic Asian longhorned beetle *Anoplophora glabripennis*. *Agric. For. Entomol.* 11: 359–375.
- Li, S., Z. Zhang, J. Luo, C. He, Y. Pu, and X. An. 2005. Progress and strategies in cross breeding of poplars in China. *For. Stud. China* 7: 54–80.
- Liebholt, A. M., D. G. McCullough, L. M. Blackburn, S. J. Frankel, B. Von Holle, J. E. Aukema. 2013. A highly aggregated geographical distribution of forest pest invasions in the USA. *Divers. Distrib.* 19: 1208–1216. doi: 10.1111/ddi.12112.
- Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences and control. *Ecol. Appl.* 10: 689–710.
- Markham, C., and B. Reardon. 2013. Asian Longhorned beetle (ALB), *Anoplophora glabripennis*, eradication program, p. 99. In K. A. McManus and K. W. Gottschalk (eds.), Proceedings of the 23rd U.S. Department of Agriculture interagency research forum on invasive species 2012, 10–13 January 2012, Annapolis, MD. Gen. Tech. Rep. NRS-P-114. Newtown Square, PA: U.S. Department of Agriculture, Forest Service, Northern Research Station.
- Meng, P. 2014. Where's that smell? Trapping and sensory biology of the Asian longhorned beetle, *Anoplophora glabripennis*. M.S. Thesis, The Pennsylvania State University, State College, PA.
- Nehme, M. E., M. A. Keena, A. Zhang, T. C. Baker, and K. Hoover. 2009. Attraction of *Anoplophora glabripennis* to male-produced pheromone and plant volatiles. *Environ. Entomol.* 38:1745–1755.
- Nehme, M. E., M. A. Keena, A. Zhang, T. C. Baker, Z. Xu, and K. Hoover. 2010. Evaluating the use of male-produced pheromone components and plant volatiles in two trap designs to monitor *Anoplophora glabripennis*. *Environ. Entomol.* 39: 169–176.
- Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien invasive species in the United States. *Ecol. Econ.* 52: 273–288.
- R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 (<http://www.R-project.org/>) (accessed 26 February 2014).
- Reddy, G.V.P., R. Fettköther, U. Noldt, and K. Dettner. 2005. Enhancement of attraction and trap catches of the old-house borer, *Hylotrupes bajulus* (Coleoptera: Cerambycidae), by combination of male sex pheromone and monoterpenes. *Pest Manage. Sci.* 61: 699–704.
- Simberloff, D., and M. Rejmanek. 2011. Encyclopedia of Biological Invasions. University of California Press, Berkeley and Los Angeles, CA. p. 765.
- The Mathworks Inc. 2012. Global Optimization Toolbox: User's Guide (R2012b). ([www.mathworks.com/help/pdf\\_doc/gads/gads\\_tb.pdf](http://www.mathworks.com/help/pdf_doc/gads/gads_tb.pdf)) (accessed November, 2012).
- (USDA-APHIS) U.S. Department of Agriculture–Animal Plant Health Inspection Service. 2013a. News release: Federal and State Officials Announce Tree Survey Efforts in Babylon Township, New York Due to the Discovery of Asian Longhorned Beetle. ([http://www.aphis.usda.gov/newsroom/2013/09/ny\\_babylon\\_township\\_alb.shtml](http://www.aphis.usda.gov/newsroom/2013/09/ny_babylon_township_alb.shtml)) (accessed 13 May 2014).
- (USDA-APHIS) U.S. Department of Agriculture–Animal Plant Health Inspection Service. 2013b. Asian longhorned beetle cooperative eradication program in clermont county, Ohio. ([http://www.aphis.usda.gov/plant\\_health/ea/downloads/2012/ALB-OHclermontCountyRevisedEA.pdf](http://www.aphis.usda.gov/plant_health/ea/downloads/2012/ALB-OHclermontCountyRevisedEA.pdf)) (accessed 13 May 2014).
- (USDA-APHIS) U.S. Department of Agriculture–Animal Plant Health Inspection Service. 2014a. Federal order adding portions of the towns of Oyster Bay, Huntington, and Babylon on Long Island, New York, to the Asian longhorned beetle regulated area. ([http://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/asian\\_lhb/downloads/DA-2014-20.pdf](http://www.aphis.usda.gov/plant_health/plant_pest_info/asian_lhb/downloads/DA-2014-20.pdf)) (accessed 13 May 2014).

- (USDA–APHIS) U.S. Department of Agriculture–Animal Plant Health Inspection Service. 2014b. ALB eNewsletter April 30, 2014 edition. (<http://beetlebusters.info/newsletter/>) (accessed 13 May 2014).
- Williams, D. W., H. P. Lee, and I. K. Kim. 2004. Distribution and abundance of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in natural Acer stands in South Korea. *Environ. Entomol.* 33: 540–545.
- Zhang, A. J., J. E. Oliver, J. R. Aldrich, B. D. Wang, and V. C. Mastro. 2002. Stimulatory beetle volatiles for the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky). *Z. Naturforsch. C.* 57: 553–558.
- Zhang, A. J., J. E. Oliver, K. Chauhan, B. G. Zhao, L. Q. Xia, and Z. C. Xu. 2003. Evidence for contact sex recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Naturwissenschaften* 90: 410–413.
- Zhang, F., X. Wu, Y. Li, and Y. Jin. 2007. Volatiles on behavior responses of *Anoplophora glabripennis* by the supercritical carbon dioxide extraction of *Acer mono*. *Scientia Silvae Sinicae* 43: 146–150.

*Received 26 February 2014; accepted 20 May 2014.*

---